PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 16894	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/CA2004/000998	International filing date *(day/month/year)* 08.07.2004	Priority date *(day/month/year)* 08.07.2003
International Patent Classification (IPC) or national classification and IPC C12N5/06, A01K67/027		
Applicant MCGILL UNIVERSITY et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06.05.2005	21.10.2005
Name and mailing address of the International preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Brouns, G Telephone No. +31 70 340-3789

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/CA2004/000998

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-33 as originally filed

Claims, Numbers

1-26 filed with the demand

Drawings, Sheets

1/14-14/14 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☒ the claims, Nos. 4,16,17
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1,2,4,5,16-21
	No:	Claims	3,6-15,22-26
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-26
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	-

2. Citations and explanations (Rule 70.7):

see separate sheet

The present application relates to embryonic stem (ES) cells obtainable from crosses between C57BL/6 and 129 inbred mouse (sub)strains. Some of said ES cells comprise a transgene docking site for introduction of a single copy introduction of a transgene.
It has been shown that some of the ES cells derived from three inbred strains had the potential to give rise to chimeras with 100% ES cell derived coat color using the morula aggregation technique of generating embryos, indicating good development potential.

Re Item I

Basis of the report

Amended claims 4, 16 and 17, do not fulfil the requirement of Article 34(2)(b) PCT since no basis may be found for said amended claims:
In claim 4, the term 'X-linked' has been removed, whereas the description relates to X-linked HPRT.
Neither the description nor the claims as originally filed disclose a method for preparing mouse ES cells having good developmental potential, performing multiple generation of breeding including a combination of 'at least one cross and at least one backcross' (claims 16 and 17).
The amendments in claims 4, 16 and 17 have not been taken into consideration for this International Preliminary Examination Report.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) Reference is made to the following documents:

D1: EGGAN KEVIN ET AL: "Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation" PROC. NATL. ACAD. SCI. USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 98, no. 11, 22 May 2001 (2001-05-22), pages 6209-6214

D2: YAGI TAKESHI ET AL: "A novel ES cell line, TT2, with high germline-differentiating potency" ANALYTICAL BIOCHEMISTRY, vol. 214, no. 1, 1993, pages 70-76

D3: JASIN ET AL: "Targeted transgenesis" PROC. NATL. ACAD. SCI. USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 93, no. 17, August 1996 (1996-08), pages 8804-8808

NOVELTY (Article 33(2) PCT)

2.1) D1 discloses ES cells derived from various crosses between two different inbred mouse strains, including C57Bl/6 and a 129 substrain, and shows that said F_1 ES cell clones can give rise to embryos entirely (100%) derived from said ES cells, when injected into tetraploid blastocysts and subsequently transferred to recipient females (D1, table 4; page 6213, right hand column, paragraph 1). Some of the ES cells described were derived from mouse strains carrying a transgene docking site, the *Rosa26* locus, and mice have been generated from said cells after targeting of said *Rosa26* locus (D1, table 4).

It has been noted that D1 does not show that the ES cells derived from the hybrid mice compete successfully with pre-existing inner cell mass cells, and their derivatives, when injected into a normal blastocyst. However, there are no **technical features** that allow the skilled person to discriminate between the ES cells disclosed in D1 and the ES cells of claim 3 or 22, therefore they are considered to be implicitly identical.
If the ES cells disclosed in D1 are not suitable to practise the present invention, it seems that not all ES cells falling under the scope of claim 3 would solve the problem posed, hence said claim would lack support.
In conclusion, D1 anticipates the subject-matter of claims 3, 6-11 and 22-26 (Article 33(2) PCT).

2.2) In addition, claims 12-15 lack novelty because a product is not rendered novel merely by the fact that it is produced by means of a new process (PCT Guidelines A5.26[1]), and because there are no technical features present in claims 12 -15 that allow the skilled person to distinguish between an ES cell derived mouse or transgenic mouse of said claims from (transgenic) mice obtained by crossing two or three inbred strains of mice known in the art (Article 33(2) PCT).

2.3) The subject-matter of claims 1, 2, 4, 5 and 16-21 is not disclosed in the prior art, therefore said claims are novel (Article 33(2) PCT).

INVENTIVE STEP (Article 33(3) PCT)

3.1) The document D2 is regarded as being the closest prior art to the subject-matter of claim 1, and discloses (D2, table 1): ES cells derived from a cross between two different inbred mouse strains, that give rise to chimeras that are 100% ES cell derived upon injection of the ES cells into eight-cell stage embryos.
From this the subject-matter of claim 1 differs in that **three** inbred (sub)strains have been used to generate ES cells having maximal heterosis and related development potential.

The problem to be solved by the present invention may therefore be regarded as the provision of further ES cells with maximal heterosis.

No surprising effect has been indicated of the use ES cells derived from a cross between three different inbred mouse (sub)strains compared to ES cells derived from a cross between two inbred mouse strains for the ability of said ES cells to contribute at a high percentage to the embryos generated from said ES cells. It is known in the art that heterozygous ES cells can give rise to entirely ES cell derived embryos (D1, table 4; D2, table 1) and the present application does not provide evidence for a **technical contribution** of the genetic information of a third inbred (sub)strain to heterosis and related development potential.
Therefore no inventive step may be acknowledged for the subject-matter of claim 1 (Article 33(3) PCT).

3.2) A method for preparing mouse ES cells having good development potential by mating a male of a first inbred strain with a female of a second inbred strain is well known in the art (D1, D2). From this, the subject-matter of claim 16 differs in that in addition multiple generations of breeding including crosses and backcrosses are performed with the offspring of aforementioned first mating.
The subject-matter of claim 16 thus provides a further method for preparing ES cells having good development potential.
The further breeding steps will change the relative contribution of the genetic information of

both inbred (sub)strains to the ES cells. However, the application fails to indicate what effect on the development potential of the ES cells is achieved by changing the ratio of the genetic information of both inbred (sub)strains, or what ratios would be suitable to practise the invention.
Similarly, no comparative example has been provided to demonstrate that a method of combining the genetic information derived from any three inbred (sub)strains (claim 17) results in ES cells with better development potential than the hybrid ES cells known in the art. Therefore, no technical effect has been demonstrated for the additional breeding steps or the introduction of genetic information from a third inbred (sub)strain, and no inventive step has been acknowledged for the subject-matter of claim 16 and 17.

3.3) Dependent claims 2, 4, 5 and 18-21 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, the reasons being as follows:
the use of a specific sequence allowing introduction of a single copy insertion of a transgene in such a manner that does not disrupt endogenous genes, such as a deletion mutant of an X-linked hypoxanthine phosphoribosyltransferase gene or a sequence comprising a loxP site is well known (D3).

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THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A preparation of non-inbred mouse embryonic stem (ES) cells that comprise alleles derived from at least three different inbred mouse strains, wherein the ES cells have good developmental potential and successfully compete with pre-existing inner cell mass cells, and their derivatives, when injected into a normal blastocyst.

2. The non-inbred ES cell preparation according to claim 1, wherein the ES cells additionally comprise a transgene docking site.

3. A preparation of non-inbred mouse embryonic stem (ES) cells that comprise alleles derived from at least two different inbred mouse strains and a transgene docking site, wherein the ES cells have good developmental potential and successfully compete with pre-existing inner cell mass cells, and their derivatives, when injected into a normal blastocyst.

4. The non-inbred ES cell preparation according to claim 2 or 3, wherein the transgene docking site is a deletion mutant of a hypoxanthine phosphoribosyltransferase (HPRT) gene.

5. The non-inbred ES cell preparation according to claim 2 or 3, wherein the transgene docking site comprises a *loxP* site.

6. The non-inbred ES cell preparation according to any one of claims 1 – 5, wherein chimeras derived from the ES cells exhibit greater than 50% ES cell contribution.

7. The non-inbred ES cell preparation according to claim 6, wherein chimeras derived from the ES cells exhibit greater than 90% ES cell contribution.

8. The non-inbred ES cell preparation according to claim 7, wherein chimeras derived from the ES cells exhibit about 100% ES cell contribution.

9. A method for producing an ES cell-derived mouse comprising the steps of:

   (a) introducing a non-inbred mouse ES cell preparation according to any one of claims 1 - 8 into a normal mouse blastocyst or a tetraploid mouse

blastocyst or aggregating a non-inbred mouse ES cell preparation according to any one of claims 1 – 8 with one or more pre-implantation embryos under conditions that result in production of at least one embryo;

(b) transferring the resulting embryo(s) into an appropriate foster mother; and

(c) maintaining the foster mother under conditions that result in development of live offspring.

10. A method for producing an ES cell-derived, transgenic mouse comprising the steps of:

(a) introducing one or more transgenic sequences into non-inbred mouse ES cells of an ES cell preparation according to any one of claims 2 or 4 - 8;

(b) maintaining the ES cells under conditions that result in homologous recombination at the transgene docking site such that the one or more transgenic sequences are incorporated in the genome of the ES cells;

(c) introducing the resultant recombinant ES cells into normal blastocyst(s) or tetraploid blastocyst(s) or said recombinant ES cells with one or more pre-implantation embryos, under conditions that result in production of at least one embryo;

(d) transferring the resulting embryo(s) into an appropriate foster mother; and

(e) maintaining the foster mother under conditions that result in development of live offspring, wherein the ES cells have good developmental potential.

11. A method for producing an ES cell-derived, gene targeted mouse comprising the steps of:

(a) performing a genetic alteration or mutation of one or more genes or parts of genes in non-inbred mouse ES cells of an ES cell preparation according to any one of claims 1 - 8;

(b) maintaining the ES cells under conditions that result in homologous recombination such that the knock-out is incorporated in the genome of the ES cells;

(c) introducing the resultant recombinant ES cells into normal blastocyst(s) or tetraploid blastocyst(s) or said recombinant ES cells with one or more pre-implantation embryos, under conditions that result in production of at least one embryo;

(d) transferring the resulting embryo(s) into an appropriate foster mother; and

(e) maintaining the foster mother under conditions that result in development of live offspring, wherein the ES cells have good developmental potential.

12. The method according to any one of claims 9, 10 or 11, wherein the appropriate foster mother is, a pseudopregnant female mouse.

13. An ES cell-derived mouse that is prepared according to the method of any one of claims 9 – 12.

14. The mouse according to claim 13, which is a transgene bearing mouse.

15. The mouse according to claim 13, which is a genetically altered or mutated mouse.

16. A method for preparing mouse embryonic stem cells having good developmental potential that comprises the steps of:

(a) mating a female mouse of a first inbred mouse strain with a male mouse of a second inbred mouse strain, wherein the first and the second mouse strains are different;

(b) performing multiple generations of breeding including a combination of at least one cross and at least one backcross from offspring obtained from the mating between the female mouse and the male mouse in step (a);

(c) recovering blastocysts from a mouse obtained following the multiple generations of breeding performed in step (b); and

(d) deriving embryonic stem cells from the inner cell masses of said blastocysts.

17. A method for preparing mouse embryonic stem cells having good developmental potential that comprises the steps of:

(a) mating a female mouse of a first inbred mouse strain with a male mouse of a second inbred mouse strain, wherein the first and the second mouse strains are different;

(b) mating an offspring of the mating of step (a) or an offspring of a subsequent generation with a mouse of a third inbred mouse strain;

(c) performing multiple generations of breeding including a combination of at least one cross and at least one backcross from offspring obtained from the mating of step (b);

(d) recovering blastocysts from a mouse obtained following the multiple generations of breeding performed in step (c) and

(e) deriving embryonic stem cells from the inner cell masses of said blastocysts.

18. The method according to claim 16 or 17, wherein the multiple generations of breeding comprises a combination of 5 or 6 crosses and backcrosses.

19. The method according to any one of claims 16 – 18, wherein at least one of the inbred mouse strains contains a transgene docking site.

20. The method according to claim 19, wherein the transgene docking site is a deletion mutant of a hypoxanthine phosphoribosyltransferase (HPRT) gene.

21. The method according to claim 19, wherein the transgene docking site comprises a *loxP* site.

22. A non-inbred embryonic stem (ES) cell preparation obtained by the method of any one of claims 16 – 21.

23. Use of the ES cell preparation according to any one of claims 1 – 8 or 22 for producing an ES cell derived mouse.

24. Use of the ES cell preparation according to any one of claims 1 – 8 or 22 for producing an ES cell derived genetically modified mouse.

25. The use according to claim 24, wherein said genetically modified mouse is a transgenic mouse.

26. The use according to claim 24, wherein said genetically modified mouse comprises a genetic alteration or mutation.